By Kyle Albert & Josh Mixdorf

ESTROGEN DETECTION BY LUMINANCE

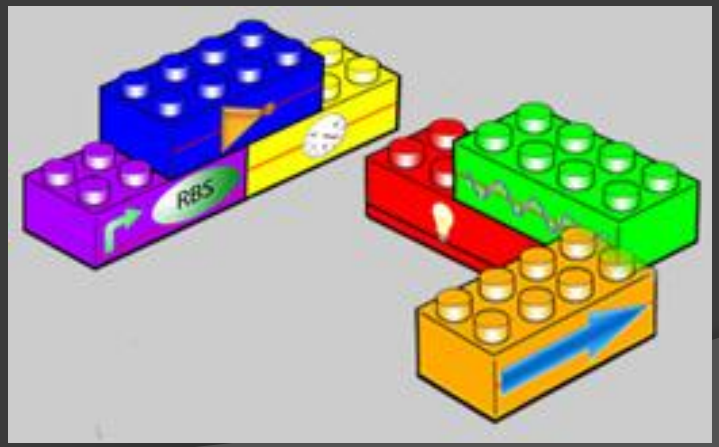
Why

- Uncontrolled levels of estrogen in waste water treatment plants is a growing problem.
- The high levels of estrogen have been found to cause feminization of fish that are exposed to it.
- More studies are showing that it is having an effect on humans as well causing an increase in XXY genotypes in prenatal babies.

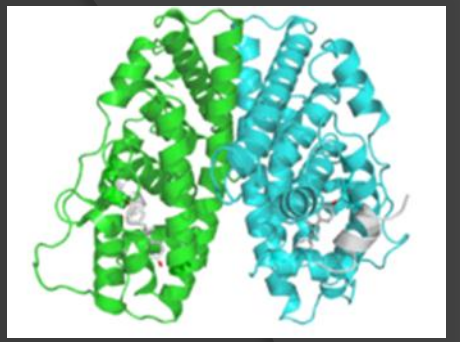
Goals

- To detect the presence of estrogen.
- To iluminate *E. coli* as a detection method.

Parts



BBa_K123003



- When estrogen or an estrogen like compound is present the receptor binds to it and forms a homodimer.
- This homodimer is then able to bind to an Estrogen Responsive Element (ERE BBA_K123002).

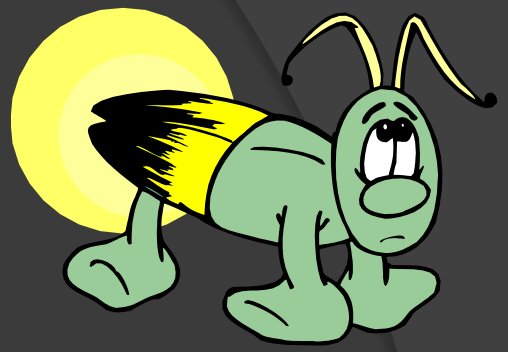
BBa_K123002

- LacIQ ERE TetR
- We believe that in conjunction with another part (Bba_K123003 ER) ERE will detect estrogen.
- We will use BBa_K123003, to bind to the ERE to act as our promoter.

BBa_J61139

- Ribosome binding site
- We will use the same coding sequence as this part but will buy it as just a stand of DNA instead of using a biobrick.

BBa_I712019



- Firefly luciferase
- Part is cloned into pSBAK3 vector with terminator.
- Includes start and stop codon.

BBa_B1006

- Terminator
- Bidirectional, with the reverse estimated to be more effective than the forward.
- We will include this terminator as a back up incase a problem occurs with stop codon from BBa_I712019.
- We will also buy the same DNA code sequence for this part instead of using a biobrick.

Order of Parts Assembly

How We Will Accomplish This

- Step 1: Join the BioBricks in accordance with the manual
  1. Join BBa_K123003 with BBa_K123002
  2. Then add the RBS DNA with the EX prefix and S suffix to the BBa_I712019 with the X prefix and the SP suffix
  3. Then add the newly formed part with an EX prefix and the S suffix to the Terminator DNA that has X prefix and SP suffix
  4. Now combine all parts specified above together: step one's part with a EX prefix and a S suffix to step three's part with a X prefix and a SP suffix

- Step 2: Introduce the new strand of DNA into a vector
- Step 3: Using vector insert DNA into *E. coli*
- Step 4: Grow the *E. coli* on an ager dish infused with estrogen and ampicillin
- Step 5: If the bacteria grow and light up on the estrogen plate and not the ampicillin only plate we have succeeded

- Step 6: Once the strand has been tested and found to work we will then test variable concentrations of estrogen to find what needed to initiate and maintain the ER